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## 117
## Isolation and characterization of bacteriophages against equine pathogens-novel phages revealed as phage therapy candidates

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A role for bacteriophage therapy was envisaged early last century; however, due to discovery of the antimicrobials, it fell out of research interest. Currently, bacteriophages are resurfacing as an alternative to antimicrobials in order to overcome the increasing incidence of antimicrobial resistance. Here, we report isolation of bacteriophages against *Escherichia coli, Shigella spp., Aeromonas hydrophila* and *Citrobacter sedlakii* isolates of equine origin. Phages were isolated from equine farm soil and sewage samples. For enrichment, sample aliquot was incubated overnight with host bacteria at 37°C with vigorous shaking. The crude lysate obtained was centrifuged and filtered and the presence of any phage in the suspension was detected by agar overlay technique. Appropriate dilutions of enriched samples were plated to obtain individual plaques and the most dominant plaque was transferred into SM buffer, serially diluted and plated for plaque re-isolation three times to ensure purity, followed by large scale preparation of phage stocks. Any host nucleic acids was degraded using pancreatic DNaseI and RNase and bacteriophage particles were precipitated using PEG8000. Phage titre was determined by plaque assay and phage concentrates were accessioned in the Veterinary Type Culture Collection (VTCC) repository. The phage concentrates were visualized by transmission electron microscopy (TEM). The temperature stability of bacteriophages was checked after incubating phage concentrates over the range of 4°C - 80°C temperature for one hour.
A clear single plaque was obtained on nutrient agar against *Shigella spp.* and after purification and concentration, its analysis by electron microscopy revealed presence of multiple phages belonging to families *Myoviridae, Siphoviridae* and *Podoviridae*. However in case of Citrobacter sedlakii, a Siphoviridae phage (VTCCBPA61) with dimensions: 60 nm x 650 nm was observed. Against a pathogenic *A. hydrophila* isolate of equine origin (expressing aerolysin gene), a Myoviridae phage (VTCCBPA6) was isolated with dimensions: 62 nm x 138 nm. Against *E. coli* of equine origin, a *Myoviridae* phage (VTCCBPA9) of dimensions: 86 nm x 100 nm was obtained. Bacteriophage VTCCPBA61 against *C. sedlakii* was found to completely lose its biological activity at 65°C in vitro however the group of phages against *Shigella spp.* were found to be stable upto temperature as high as 80°C. Thus we demonstrated the basic biological characteristics of phages, and some novel ones (such as against *A. hyrophila* and *C. sedlakii*) bacterial isolates of equine origin which have never been reported till now. These lytic phages could find potential in phage therapy, as biocides, in biosensors and in phage ligand technology and are being explored by us further to depict their therapeutic value in small animal model. As more studies are reporting safety, tolerance and efficacy of phage therapy in humans and animals, their use in phage therapy has a promising future as an emerging alternative to chemical agents.

# Posters

## 006
## Microbiological investigation into the cause of Equine Grass Sickness

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Despite intensive research for more than a hundred years, the cause of Equine Grass Sickness (EGS), has not been found yet. However, it is believed that neurotoxins produced by the bacterium *Clostridium (C) botulinum* Type C (BoNT) are responsible for the disease. Horse challenge experiments conducted at Central Veterinary Research Laboratory (CVRL), Dubai with *C. botulinum* Type C toxins as well as with the pathogen itself failed to produce clinical signs of EGS but resulted in the induction of neuromuscular classical botulism. Therefore, we believe that C. botulinum Type C is not the sole agent for EGS and started detailed investigations of the anaerobic flora in the ileal and caecal contents of 2 EGS affected horses and in a soil sample collected from an EGS affected paddock. Samples were processed by special culture and enrichment techniques and the isolated anaerobes were tested for toxin production for four days and their lethality in a mouse bioassay. In total twenty different anaerobes were isolated from the 3 samples. Few anaerobes were isolated in common from ileum, caecum and soil samples, except for *C. perfringens* with α and β2 gene. Also except for C. perfringens only soil isolates were positive in the mouse bioassay. Strains identified as *C. perfringens* (α toxin producer, α toxin and β2gene possessing), *C. subterminale*, and *C. septicum* were lethal for mice. *C. perfringens* strains carrying β-2 gene along with the ability to produce α toxin, killed mice within 24 hours and the mice showed typical wasp-waist appearance as seen with botulinum toxin. The toxigenic strains were found to yield toxin on different days of incubation.

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## 017
## Equine grass sickness, but not botulism, causes autonomic and enteric neurodegeneration and increases SNARE protein expression within neuronal perikarya

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